

to take place uniformly but sufficiently short to preserve viability of the sperm;

b) passing the sperm into an electrically conductive and isotonic viability-supporting sheath fluid to form a suspension of sperm which are caused to flow singly in a stream of sheath fluid;

c) passing the sheath fluid containing the sperm before an excitation light source causing the stained DNA to fluoresce;

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d) passing the sheath fluid containing the sperm through both a means for detecting the fluorescence of the stained DNA and also a cell sorting means, the means for detecting fluorescence having at least two detectors arranged such that a first detector determines the orientation of sperm on the basis of magnitude of fluorescence and controls a second detector to measure the DNA content of sperm on the basis of magnitude of fluorescence of those sperm that have been determined to be in a preselected orientation;

e) selecting by said cell sorting means the sperm having a DNA content corresponding to a desired chromosome which will produce a desired gender of offspring, and separating the selected sperm from nonselected sperm; and

f) collecting the selected sperm in a viability-supporting collecting fluid.

10. The method of Claim 9, wherein said mammal is a rabbit.
11. The method of Claim 9, wherein said mammal is a swine.
12. The method of Claim 9, wherein said mammal is a bovine.
13. The method of Claim 9, wherein said dye is bisbenzimidazole H33342 fluorochrome.
14. The method of Claim 9, wherein said incubation is at a temperature of about 39° C for a period of about 1 hr.
15. The method of Claim 9, wherein said incubation is at a temperature of about 35° C for a period of about 1 hr.
16. The method of Claim 9, wherein said incubation is at a temperature of about 30° C for about 1.5 hr.
17. The method of Claim 9, wherein said sheath fluid is phosphate-buffered saline solution, the solution also containing 0.1% bovine serum albumin to enhance sperm viability.

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18. The method of Claim 9, wherein said collecting fluid is modified test egg yolk extender.

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19. The method of Claim 9, wherein said sperm are hydrodynamically oriented in the flow of sheath fluid prior to being passed before said light source.

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20. The method of Claim 9, wherein said sperm are hydrodynamically oriented in the flow of sheath fluid by passing the fluid in a narrow stream through and out of a bevelled injection tip prior to being passed before said light source.

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21. A method to preselect the sex of mammalian offspring comprising:

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a) sorting sperm according to the method of Claim 9; and
b) inseminating a female mammal of the same species as the male mammal with the selected sperm in the collecting fluid.

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22. A method to preselect the sex of mammalian offspring comprising:

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a) sorting sperm according to the method of Claim 9; and
b) fertilizing an egg obtained from a female mammal of the same species as the male mammal with the selected sperm in the collecting fluid.

15 ~~23~~. The method of ~~Claim 9~~, further comprising eliminating sperm which are not properly oriented with an electronic gating system before sorting by said cell sorting means.

16 ~~24~~. The method of ~~Claim 9~~, wherein the flow of sperm through the cell sorting means is regulated by an ultrasonic transducer.

17 ~~25~~. The method of ~~Claim 9~~, wherein said sperm are sorted on the basis of X- or Y-chromosome DNA content with about 90% efficiency.

18 ~~26~~. The method of ~~Claim 9~~, wherein said sperm are hydrodynamically oriented in the flow of sheath fluid and sperm which are not properly oriented are eliminated by an electronic gating system prior to being passed before said light source.

27. A method to preselect the sex of mammalian offspring comprising:

a) staining sperm collected from a male mammal with a fluorescent dye capable of selectively staining DNA in living cells by incubating sperm with the dye at a temperature in the range of about 30°-39° C for a period of time sufficiently long for staining to take place uniformly but sufficiently short to preserve viability of the sperm;

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b) passing the sperm into an electrically conductive and isotonic viability-supporting sheath fluid to form a suspension of sperm which are caused to flow singly in a stream of sheath fluid;

c) passing the sheath fluid containing the sperm before an excitation light source causing the stained DNA to fluoresce;

d) passing the sheath fluid containing the sperm through both a means for detecting the fluorescence of the stained DNA and also a cell sorting means to measure the DNA content of the sperm on the basis of magnitude of fluorescence of the sperm;

e) selecting by said cell sorting means the sperm having a DNA content corresponding to a desired chromosome which will produce the desired gender of offspring, and separating the selected sperm from nonselected sperm; and

f) collecting the selected sperm in a viability-supporting collecting fluid.

REMARKS

Claims 9-27 are now in the case.

Claims 1-8 have been cancelled.

Applicant submits that Claims 9-27 distinguish over the prior art of record, particularly those references applied against the claims in parent application Serial No. 07/349,669, for the following reasons.